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ENCAPSULATION OF COS MOLECULES IN HMWNC FOR ANTIOXIDANT ACTIVITY

Chandrika Ahirwal
Research Scholar Sunrise University, Alwar

Dr. Narendra Singh
Professor, Sunrise University, Alwar

ABSTRACT

After demineralization and deproteinization of pulverized crab shells, chitin was obtained in abundance, and this was used to prepare high molecular weight nanocrystal line chitosan (HMWNC) with Mw of 350 kDa and 66.92% DDA, which was then used to encapsulate commercially available chitosan oligosaccharide (COS) with average Mw 3000 Da and 87% DDA. It was expected that increasing COS's bioavailability in target cells would be possible via encapsulating it in HMWC. Antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was seen in vitro for the HMWNC-encapsulated chitosan oligosaccharide (COS-HMWNC) but not for the other samples (IC50 = 512.614.4 g/mL). The antioxidant activity of chitosan increased as its molecular weight decreased. The results of the research demonstrated that the antioxidant activity of COS might be improved by encapsulating COS molecules in HMWNC.

Keywords: -Antioxidant activity, chitin, chitosan oligosaccharide, high molecular weight nanocrystal line chitosan, encapsulation

I. INTRODUCTION

Chitin is a homopolymer of 2-acetamido-2-deoxy- -D-glucose monomers joined via (14) links, and it is the second most prevalent natural polymer after cellulose. Crustacean shells, the primary commercial source of chitin, are composed of a variety of minerals and other components, including 15–40% chitin, 20–40% protein, and 20–50% calcium carbonate and magnesium carbonate. Demineralization with hydrochloric acid and deproteinization with alkali are used to extract chitin from the exoskeleton of crustaceans. Chitosan is a copolymer of N-acetyl and deacetyl -glucosamine (C6H11O4N) units, and it is a partly deacetylate derivative of chitin. Because an amino group in its backbone is protonated, chitosan is a cationic polysaccharide. Figure 1) and a natural product with some synthetic components and several potential uses.

Chitosan is a substance utilized in the targeted administration of pharmaceuticals due to its adsorbent, pharmaceutical excipient, permeability enhancing, and hemostatic properties. Activities against microbes, cancer, fungus, free radicals, and obesity have been observed. Both the degree of deacetylation (DDA) and the molecular weight (Mw) of chitosan have significant effects on its functional qualities, including its biocompatibility and bioactivity. Natural antioxidant high molecular weight chitosan (HMWC) has been suggested to have promising biological uses. When nano chitosan is synthesized as biocompatible polymeric nanoparticles, its cellular absorption and persistence in the blood are extended due to increased extravasation and passive targeting. The charge of the Carrier is crucial to the absorption of the encapsulated drug by cells. Moving toward the negatively charged cell membranes is the cationic carrier.

II. REVIEW OF LITERARURE

Arfeen, Minhajul&Bharatam, Prasad (2012) Glycogen Synthase Kinase-3 (GSK-3) is a multifunctional serine/threonine kinase that acts constitutively and has been implicated in several physiological processes. It is thus a potential target for the therapy of various disorders, including Type-II diabetes and Alzheimer's. The main obstacle was designing a GSK-3 β selective inhibitor, which prompted the employment of logical strategies such structure-based methodologies (molecular docking) and ligand-based strategies (QSAR, pharmacophore mapping) investigations. These techniques provide designers of potential GSK-3 β inhibitors information into the interactions between enzymes and ligands as well as the structure-activity connection of various sets of molecules. Studies using molecular dynamic modelling have also been carried out to address important difficulties, such as the particular need for GSK-3 to phosphorylate its substrate at P+4. Additionally, an allosteric site has been identified, where the peptide's binding results in the stabilisation of the activation loop, improving the catalysis of enzymes. The development of clinically effective selective GSK-3 β inhibitors is being aided by these investigations. In this paper, we provide an overview of current initiatives and potential future directions for the development of selective GSK-3 β inhibitors.

Liu, Ping, Zhao-Peng Liu, Xiao-Long Shi, Jing-De Wu, and Ping Liu. (2019). A number of new GSK-3 inhibitors containing the 2,3-diaminopyridine moiety were developed and synthesised to target the various aspects of Alzheimer's disease (AD). The amide derivatives 5a-f had a mediocre amount of effectiveness against GSK-3 β and had poor capacity to chelate Cu 2+, Zn 2+, and Al 3+. The imine derivatives 9a, 9b, and 9e were effective GSK-3 β inhibitors as well as specific chelators for Cu 2+ and Al 3+. The 1,2-diamine derivatives 10a-e were effective metal chelators but lost or diminished their ability to inhibit GSK-3 β. In vitro, compounds 9a, 9b, and 9e, particularly 9b, demonstrated strong ROS generation inhibition, Cu 2+ -induced A aggregation inhibition, Cu 2+ -A complex disaggregation, and antioxidant properties. Compounds 9a, 9b, and 9e have the ability to prevent tau protein phosphorylation in cells and shield nerve cells from Cu^{2+} -Aβ 1–42 and H 2 O 2 -induced cell damage. Additionally, compound 9b was projected to possess drug-like qualities and the capacity to breach the BBB. As a result, compound 9b may

serve as an excellent starting point for the creation of new GSK-3 β inhibitors that target various aspects of AD.

Stefan Berg, Margareta Bergh, Sven Hellberg, Katharina Högdin, Yvonne Lo-Alfredsson, TatjanaWeigelt, Mats Ormö, YafengXue, Julie Tucker, Julie Neelissen, Eva Jerning, and Yvonne Nilsson. (2012). Theproline-directed serine/threonine kinase glycogen synthase kinase-3, also known as tau phosphorylating kinase, was first discovered because of its function in the metabolism of glycogen. In the brain of people with Alzheimer's disease (AD), active forms of GSK3 are seen around neurofibrillary tangles and dystrophic neuritis. We have created highly potent and selective inhibitors with cellular efficacy and blood-brain barrier penetrance by using a high throughput screening (HTS) approach to look for new chemical series and cocrystallization of key analogues to guide the optimisation and synthesis of our pyrazine series. The inhibitors are appropriate for in vivo effectiveness testing and might provide a fresh approach to treating Alzheimer's.

Dan, Nguyen &Quang, Hoang & Truong, Vuong&Nghi, Do &Cuong, Nguyen &Cuong, To &QuocToan, Tran & Bach, Long Giang&Anh, Nguyen & Mai, Nguyen &Thi Lan, Ngo &Chinh, Luu& Pham, Quan. (2020) Indirubin-3′-oxime was modified with chalcone and amine components to produce 15 novel, very productive derivatives. Through the use of X-ray crystallography, 1D, 2D-NMR, and HR-MS(ESI) spectra, novel derivative structures were also clarified. For cytotoxic activity against four human cancer cell lines (HepG2, LU-1, SW480, and HL-60) and one human normal kidney cell line (HEK-293), all developed compounds were tested. The most pronounced cytotoxicity was shown by compound 6f, whereas compounds 6e, 6h, and 6l were more severely harmful to cancer cell lines than to normal cells. The GSK-3 β enzyme was used in molecular docking experiments to further analyse these novel compounds. The majority of the compounds showed potential inhibitory action against GSK-3 β, according to docking studies, with recognisable interacting residues in the binding site. The binding affinity and mechanism between ligands and the GSK-3 β enzyme were further improved using the quick pulling of ligand strategy. The computational results are anticipated to help forecast the enzyme target of the experimental inhibitors and their potential interactions, from which new cytotoxic medicines may one day be designed.

Yangping Zhou, Lijuan Zhang, Xiujuan Fu, Jiang Zhongliang, Rongsheng Tong, Jianyou Shi, and Lei Zhong. (2019). A key regulatory function is played by glycogen synthase kinase 3 (GSK3) in a number of signalling pathways, including PI3K/AKT, which is strongly linked to the emergence and growth of tumours. The majority of currently known active GSK3 inhibitors all have the same structural makeup, either a lactam ring or an amide structure. We thoroughly examined previously reported crystal-binding patterns of GSK3 small molecule inhibitor with GSK3 protein, and then designed and synthesised 17 non-reported 3,5-diamino-N-substituted benzamide compounds in an effort to identify the GSK3 β small molecule inhibitor with a novel, safe, efficient, and more straightforward synthesis method. 1H NMR, 13C NMR, and HRMS were

used to establish their structural integrity. By using MTT, chemicals that were first found to be tumor-cytotoxic were identified, and the correlations between their structure and activity were shown. According to the findings, compound 4d demonstrated noteworthy selectivity to GSK3 β and considerable tumour cytotoxicity against human colon cancer cells (HCT116). Its IC50 value was 8.3 M. Additionally, Compound 4d has weak PK characteristics and a limited ability to trigger apoptosis.

MorihisaSaitoh, Jun Kunitomo, Eiji Kimura, Yoji Hayase, Hiromi Kobayashi, Noriko Uchiyama, Tomohiro Kawamoto, Toshimasa Tanaka, Clifford Mol, Garret Textor, Gyorgy Snell, and Fumio Itoh (2009). The aberrant hyperphosphorylation of tau protein is thought to be caused by glycogen synthase kinase-3 beta (GSK-3beta), and its inhibitors are anticipated to be a viable therapeutic agent for the treatment of Alzheimer's disease. Here, we discuss a new set of oxadiazole derivatives' design, synthesis, and structure-activity interactions as GSK-3beta inhibitors. Compound 20x, one of these inhibitors, demonstrated very effective and specific inhibition of GSK-3beta in vitro, and its way of binding was identified by acquiring the X-ray co-crystal structures of 20x and GSK-3beta.

Arfeen, Minhajul&Bhagat, Shweta & Patel, Rahul & Prasad, Shivcharan& Roy, Ipsita&Chakraborti, Asit&Bharatam, Prasad. (2016) Iminothiazolidin-4-one derivatives were investigated in this study as potential selective GSK-3β inhibitors. A number of compounds were created utilising substituted thiourea, 2-bromoacetophenones, and benzaldehydes after molecular docking studies. Nine compounds having activity in the lower nano-molar range (2-85 nM) were found among the twenty-five compounds that were synthesised during this investigation after being tested in vitro against GSK-3 β. Additionally, in vitro testing against CDK-2 revealed five drugs to be GSK-3 β -selective.

III. MATERIALS AND METHODS

Materials

The fishing waste of crab shells was gathered from the Kathmandu, Nepal, market. Glacial acetic acid (Merck 99-100%), hydrochloric acid (Merck 99%), sodium hydroxide (Merck, 99%), sodium acetate (Merck), ethanol (Sigma-Aldrich, 99.80%), methanol (Sigma-Aldrich, 99.80%), chitosan oligosaccharide (Sigma-Aldrich, 87%) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) (SigmaAldrich, 95%) were used as received.

Measurements

Using a BRUKER 1 003 3610 FT-IR spectrophotometer with ATRGeXPm experiments, the powdered form's FT-IR spectra was measured in the 4000-400 cm-1 areas. We used a BRUKER AC-800 Delta 2 NMR spectrometer, polarized at 400 MHz, with 276 scans and 3.5 minutes of contact time to get the solid-state 13C-NMR spectra. Using a D8-Advance BRUKER

diffractometer with a Cu target (= 0.1541 nm) at 40 kV, powder X-Ray Diffraction (XRD) measurements were taken with a scanning scope of 2 from 0 to 60 degrees and an exposure period of 400 S. Elements were analyzed in an oven preheated to 75 degrees Celsius using a ThermoFinnigan FLASH EA 112CHNS microanalyzer and carrier gas He (140 mL/min) on a CHNS/ NCS column of PQS SS 2M 6X5 mm.

Experiments Synthesis of nanocrystalline chitosan

Crab shell powder was demineralized and deproteinized to get chitin, and then chitin was alkaline deacetylated to produce crab shell chitosan, as described in other publications [38, 39]. The technique described by Pighinelli et al. [24] for producing nanocrystalline chitosan was slightly modified. After dissolving 0.5 g of chitosan from crab shells in 100 mL of 1% acetic acid solution at 60 °C with stirring for 36 hours, 5 mL of plasticizing glycerol was added. When the mixture reached neutral pH, sodium hydroxide solution was added while stirring. After 24 hours at 5 °C, we filtered and rinsed the solution with 2 L of deionized water, and then dried the nanocrystalline chitosan at room temperature.

Encapsulation of chitosan oligosaccharide

The technique of encapsulating oregano essential oil in chitosan nanoparticles provided by Hosseini et al, was easily synthetically modified to encapsulate COS in HMWNC. After ultrasonicating in an ice bath for 5 minutes, centrifuging the opalescent solution for 30 minutes, and drying the residue obtained upon filtration at 60 °C for 36 hours, 1 mg of lyophilized nanocrystalline chitosan was added dropwise to 1 mL of chitosan oligosaccharide solution (1% (w/v) in 1% acetic acid solution) with constant stirring for half an hour. The chitosan encapsulating mass was centrifuged, and the supernatant solution was collected and kept at 4 °C.

Antioxidant assay

Slight adjustments were made to Brand William's procedure for measuring DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity. Half an hour at 37 degrees Celsius was allotted for the reaction of test compounds with the stable 1, 1-diphenyl-2-picrylhydrazyl free radical. After incubation, the multiplate reader (EPOCH2, microplate reader, Biotek) recorded the reduction in absorbance at 517 nm. Now, using the following formula, we were able to determine the extent to which the sample scavenged DPPH free radicals.

Percentage scavenging = (Ao-As)/Ao×100

whereAo= DPPH absorbance and As= DPPH free radical solution absorbance with sample extract added. As a reference point, we compared everything to quercetin (Himedia). There were three separate runs of each experiment. The concentration was shown on the x-axis and the scavenging efficiency on the y-axis in the usual way.

IV. RESULTS AND DISCUSSION

General

Crustaceans' exoskeletons and shells are composed of chitin, which is rich in minerals (mostly CaCO3) and protein. Crab shells are a rich source of chitin because, following demineralization and deproteinization, they form dazzling white crystals of chitin. The chitosan extracted from crab shells is a high molecular weight chitosan, with an average molecular weight (Mw) of 350 kDa and a degree of hydrophobicity (DDA) of 66.92%. In a solid/solvent ratio of 1:10 (w/v), 2 hours of deacetylation with 40% sodium hydroxide solution yielded a chitosan yield of 31.4%, which was close to the 32.2% reported for crab shell wastes, but higher than the 16.7% reported for 30 minutes of deacetylation with 45% sodium hydroxide solution. Figure 3a shows a crystal of commercially available COS that dissolves in water, whereas 3b shows a powder of HMWNC made from crab shell that is insoluble in water but partly soluble in dimethyl sulfoxide (DMSO).

V. CONCLUSIONS

Crab shell byproducts were processed into a semi-synthetic natural substance called nanocrystalline chitosan with a high molecular weight. Antioxidant activity was significantly improved by encapsulating commercially available chitosan oligosaccharide with this product. Increases in DDA and decreases in Mw were reported to promote the absorption of antioxidant chitosan by cells in vitro. Uptake of the chemical by cells and its subsequent scavenging of free radicals inside the cell are responsible for its antioxidant effect. Therefore, it may be generally claimed that samples higher in DDA and lower in Mw have higher cellular uptake for the net antioxidant action. Sustainable and functional antioxidants with cellular selectivity and regulated release of nano encapsulated chitosan are an open topic for future research.

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